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The effect of feeding time on the quality of metabolic control, day-to-day variability of blood glucose curves and evaluation of IGF-1 levels in cats with diabetes mellitus

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Contents

Summary

Part 1: The goal of the study was to investigate if an injection-meal-index (IMI) has a beneficial effect on metabolic control in diabetic cats. From the time of diagnosis 8 diabetic cats were regularly re-evaluated at our clinic during a study period of 24 weeks. After an adjustment period of 8 weeks, cats were fed either initially at the same time as the insulin administration for 8 weeks and then 45 min. after insulin injection for another 8 weeks or vice versa. Results showed no improvement in metabolic control with a 45-minute IMI in diabetic cats and there was no correlation between the quality of metabolic control and postprandial blood glucose concentration.

Part 2: The objective was to evaluate day-to-day variability of owner generated blood glucose curves (BGCs) in diabetic cats and compare them to BGCs performed in clinic. 7 diabetic cats of owners capable of performing blood glucose measurements at home were included. Home curves on two consecutive days and a clinic curve performed few days later were obtained on two occasions from each cat. Results of home curves were compared with each other and with the corresponding clinic curve. Differences between all parameters of home curves showed a high variation and no better results than differences between home and clinic curves indicating there is as large day-to-day variability in home curves as in clinic curves.

Part 3: The objective was to investigate if IGF-1 levels in cats with transient diabetes mellitus differ from those in cats with permanent disease. IGF-1 concentrations were measured before, 1 – 3 and 4 – 8 weeks after initiating insulin therapy. No difference in IGF-1 levels was found between cats with transient and permanent diabetes at any point in time. In both groups of cats IGF-1 concentrations were significantly lower compared to those of controls before insulin administration. After starting insulin therapy IGF-1 increased significantly in both groups. In cats with transient diabetes IGF-1 levels were not different from controls already after 1 – 3 weeks, whereas in cats with permanent diabetes it took 4 – 8 weeks.

The effect of feeding time on the quality of metabolic control in diabetic cats

1. Introduction

1.1. Literature

Diabetes mellitus is one of the most common endocrine disorders of cats. The literature indicates that 1 of every 100 to 400 cats is affected, and the incidence appears to be increasing.[1] Most diabetic cats are more than 7 years old, and the male: female incidence is about 2:1. In 80 to 95 per cent of diabetic cats, the clinical presentation corresponds to that of type 2 diabetes mellitus in humans, which is characterized by insulin resistance and beta cell dysfunction.[2] In contrast, type 1 diabetes mellitus, which usually develops during childhood or adolescence in humans, is characterized by a primary insulin shortage due to loss or destruction of beta cells.[3] Humans with type 2 diabetes do not usually need insulin treatment during the initial years of the disease. Instead, symptoms are controlled by special diets, physical exercise and oral antidiabetic drugs. However, at the time of presentation, the majority of cats are in advanced stages of the disease and have a marked, usually relative, lack of insulin.[4] The survival of these cats is dependent upon regular administration of exogenous insulin. Today, many pet owners are willing to administer insulin to prolong the life of their cat. An intermediate- or long-acting insulin, which works longer than normal insulin, is usually used in cats. The addition of zinc, which crystallizes with the insulin, or the protein protamine delays the absorption of insulin after subcutaneous injection. For adequate metabolic control in cats, intermediate- or long-lasting insulin must be administered subcutaneously every 12 hours.[5,6]

In addition to insulin and antidiabetic drug therapy, diet is of fundamental importance in human diabetic patients. For patients on fixed insulin therapy, the daily carbohydrate intake should remain constant, and for patients using multiple-dose insulin therapy, the amount of insulin administered should correspond to the amount of carbohydrate consumed at each meal. Obesity is a common problem in humans with type 2 diabetes. In these patients, the primary goal of the diet is weight reduction, which, when combined with an increase in exercise, can substantially reduce insulin resistance.[7]

In addition to the composition of the diet, the timing of insulin administration relative to the time of the meal plays an important role in glucose metabolism in insulin-dependent human diabetics. The size and timing of meals are strictly dependent on the amount and type of insulin injected beforehand. When regular or intermediate-acting insulin is administered, a predetermined period of time must elapse, the so-called injection-meal interval (IMI), before the meal is consumed. This prevents postprandial hyperglycemia, which otherwise could be caused by the delayed onset of action of the insulin, and reduces the required amount of insulin to a minimum.[8,9] In humans with type 1 diabetes, the ideal IMI was shown to be 45 minutes when a porcine intermediate-acting insulin was used.[10] Since the 1980s when recombinant human insulin became commercially available, the importance of animal-derived insulin has progressively decreased. Current insulin therapy in humans is founded on the basis-bolus principle: a human intermediate- or long-acting insulin is used as basal therapy once or multiple times during the day. In addition, regular or rapid-acting insulin is injected before each meal. Insulin analogues have been available for a number of years. These are genetically engineered forms of insulin in which the modified molecular composition results in an altered rate of absorption. For example, the rapid-acting lispro[a] is able to reduce the postprandial glucose concentration better than regular insulin, thereby substantially decreasing the required IMI.[11,12]

There are numerous studies on the ideal composition of food for diabetic dogs and cats, and a wide variety of specially formulated diets are available commercially. In contrast to the generally recommended high-fiber diets for humans, it has been determined that low-carbohydrate diets have a much more positive effect on glucose metabolism in cats. Diabetic cats that were fed low-carbohydrate, high-protein diets had a rapid improvement in clinical signs such as polydipsia, polyuria and plantigrade stance, and required less exogenous insulin or insulin therapy could even be discontinued.[13-15] Compared with high-protein diets, commercial low-protein diets result in higher postprandial insulin levels, and high-fat or high-carbohydrate diets lead to significantly higher postprandial glucose levels in healthy cats.[16] There have been few studies investigating different feeding times, and the results are controversial. Some studies suggest that half of the total caloric intake should be offered at the time of insulin administration and remain available to the cat until the next insulin injection. It is thought that those cats that are used to nibble should be allowed to eat small frequent meals because hyperglycemia is less likely to occur than with one large meal.[4,17] Other studies have postulated that, because postprandial hyperglycemia rarely occurs in cats, the timing of insulin administration and feeding do not need to be coordinated.[18,19] However, it was shown that in healthy cats, one feeding per day reduced the plasma concentration of insulin by a minimum of 40 per cent compared with feeding ad libitum. This finding indicates that one feeding per day might be advantageous, especially in diabetic cats.[20] In the literature, there are no recommendations to correlate feeding times and insulin injections or to observe an IMI.

In recent years, awareness of postprandial hyperglycemia has increased in human medicine. Previously, the primary goal of treatment of diabetic patients was normalization of the fasting glucose concentration, which was considered the critical factor in the quality of metabolic control. With time, it became apparent that the blood glucose concentration after meals played an equally important role in metabolic control and that it constitutes a risk factor for the development of late complications of the disease.[21] The most important factors affecting the postprandial glucose concentration are elevations in the absorption of glucose from the intestine, reduced insulin secretion, peripheral insulin resistance and endogenous production of glucose by the liver.[22] Elevated blood glucose levels are considered the main cause of microvascular complications and are partially responsible for the development of macrovascular complications of diabetes mellitus.[23] The risk of macrovascular complications correlates more strongly with postprandial glucose concentrations than with fasting glucose levels.[24-26] Furthermore, in a study of humans with type 2 diabetes mellitus, the concentration of glycosylated hemoglobin (Hb A_{1c}), which is the gold standard for determining long-term metabolic control, correlated better with postprandial than fasting glucose concentrations.[27]

1.2. Objectives and Hypothesis

To the author's knowledge, the effect of an IMI on postprandial glucose concentrations and metabolic control in diabetic cats has not been investigated; this was the primary goal of the present study. We assumed that, similar to human diabetics, the postprandial elevation in glucose concentration could be prevented by considering the feeding time in diabetic cats. Our hypothesis was that much better metabolic control is achieved in diabetic cats by delaying the feeding time to 45 minutes after administration of insulin. Although late complications of diabetes are of minor importance in veterinary medicine, a secondary goal of our study was to determine whether the postprandial concentration of blood glucose plays a role in the quality of metabolic control. Our second hypothesis was that the level and duration of postprandial glucose concentration substantially affect the quality of metabolic control in diabetic cats.

2. Materials and Methods

2.1. Goals

- To compare the metabolic control of diabetic cats with and without an IMI of 45 minutes
- To determine the effect of the postprandial blood glucose concentration on the metabolic control in diabetic cats

2.2. Cats

Cats that were presented to our clinic during 2003 and 2004 were used in the study. The criterion for inclusion was a diagnosis of diabetes mellitus or diabetic ketoacidosis, which was based on hyperglycemia together with glucosuria and an elevated concentration of fructosamine (>340 µmol/l). Cats that had been treated with corticosteroids or progestins up to 2 months before the onset of initial symptoms, or with insulin, and patients that had concomitant severe disease such as chronic renal insufficiency, cardiac insufficiency, severe pancreatitis, hyperthyroidism, acromegaly or Cushing's syndrome, were excluded from the study. A total of 19 cats were included in the study. During the study, 11 cats were excluded for the following reasons: transient illness (3), acromegaly (1), poor owner or cat compliance (4) and euthanasia (3). Of the 8 remaining cats, 6 were castrated males and 2 were spayed females. There were 5 domestic shorthair or longhair, 1 Siamese, 1 Burmese and 1 Carthusian cat(s). The cats ranged in age from 6 to 14 years (median 9.5 years) and weighed 3.7 to 7.7 kg (median 5.4 kg).

2.3. Groups and feeding times

The 8 cats were randomly divided into two groups. The only difference between the groups was the feeding time, which had been predetermined. 4 cats in group 1 were fed at the same time as the administration of insulin and 4 cats in group 2 were fed 45 minutes after the insulin injection. After a period of 16 weeks, the feeding times were reversed, and cats in group 1 were fed 45 minutes after the insulin injection and those in group 2 were fed at the same time as the administration of insulin. The cats were fed half their daily ration at predetermined times in the morning and evening.

2.4. Study design

From the time of diagnosis to the end of the study 24 weeks later, the cats were re-evaluated eight times at our clinic. The re-evaluations were carried out 1, 3, 6, 8, 12, 16, 20 and 24 weeks after diagnosis. During the first 8 weeks, the insulin dosage was adjusted in all the cats; these data were not used in the study (adjustment period). In the following 16 weeks, the metabolic control of all the cats was monitored at each re-evaluation (observation period). In week 16, the feeding times of the groups were reversed, so that overall, each cat was fed at the same time as the insulin injection for 8 weeks, and 45 minutes after the insulin injection for 8 weeks. The times A and B represent the re-evaluations of cats at times when they were fed at the same time as the administration of insulin, and the times C and D represent the re-evaluations of cats at times when they were fed 45 minutes after the insulin injection. For example, a cat of group 1 could be found at time A in week 12 of the study and a cat in group

2 could be found at time A in week 20 of the study.

Study protocol

Examinations	**Time**	**1***	**2***	**Event**
Presentation and diagnosis	Week 0			Random allocation to group 1 or 2
1st re-evaluation	Week 1			
2nd re-evaluation	Week 3			Introduction to home-monitoring
3rd re-evaluation	Week 6			
4th re-evaluation	Week 8			Start of observation period
5th re-evaluation	Week 12	A	C	
6th re-evaluation	Week 16	B	D	Change of feeding times
7th re-evaluation	Week 20	C	A	
8th re-evaluation	Week 24	D	B	End of study

* The numbers refer to group 1 and group 2, respectively.

2.5. Re-evaluation in the clinic

At each re-evaluation, all cats remained in our clinic for one day. On the morning of the re-evaluation, the owner administered the insulin, fed the cat according to the protocol and then brought the cat directly to our clinic. The first blood glucose concentration was determined a maximum of 2 hours after the administration of insulin. Starting in week 3, the owner determined the fasting blood glucose concentration at home before bringing the cat to our clinic. At each re-evaluation, the history was updated by asking the owner about the cat's clinical signs and their severity, and a blood sample was collected from a jugular vein. The serum concentrations of fructosamine, albumin, total protein and glucose were determined. The concentration of fructosamine was measured using an automatic analyzer[e]. Simultaneously with jugular blood collection, the glucose concentration of capillary blood was determined using a portable blood glucose meter (PBGM)[c] and a vacuum-generated lancing device.[d] These measurements were carried out every two hours until at least one hour before the next insulin injection. A blood glucose curve (BGC) was generated by plotting the measurements on a graph with the glucose concentrations on the y-axis and the time on the x-axis.

2.6. Evaluation of metabolic control and insulin dose

Metabolic control was assessed as follows:

1 = very good • no symptoms, fructosamine <400µmol/l
2 = good • no or mild symptoms, fructosamine 400 to 450µmol/l
3 = moderate • mild to moderate symptoms, fructosamine 450 to 500µmol/l
4 = moderate to poor • moderate symptoms, fructosamine 500 to 600µmol/l
5 = poor • severe symptoms, fructosamine >600µmol/l
6 = very poor • severe symptoms, fructosamine >700µmol/l

The symptomes were assessed in every re-evaluation by asking the owners about the severity of polyuria (PU) and polydipsia (PD). The cats either had no, mild, moderate or severe PU and PD. The BGC was used to determine the efficacy of the insulin dosage. The criteria used for evaluation included the level of the nadir, the difference between the maximum glucose concentration and the nadir and the time between insulin injection and occurrence of the

nadir.[4] The insulin dosage was adjusted according to the following protocol: the insulin dose was increased in cats with a nadir • 9.0 mmol/l and decreased in cats with a nadir of < 5.0 mmol/l. When the nadir was between 5.0 and 8.9 mmol/l, the insulin dose was not changed. When a problem was identified in a BGC done by the owner at home between re-evaluations, the insulin dose was adjusted accordingly.

2.7. Role of home monitoring (HM)

In diabetic cats, HM of blood glucose concentrations by the owner has been used successfully in our clinic for the past five years. Studies showed that the glucose concentrations determined using a PBGM agreed with those measured using reference methods.[28-30] In week 3 of the present study, owners were taught to use a PBGM[c] and a lancing device[d] and were asked to use the following protocol at home:

- measure the fasting blood glucose concentration twice weekly in the morning and before each re-evaluation at our clinic
- generate a BGC one week or less before the next re-evaluation

For the latter, the owner measured the blood glucose concentration every two hours for a total of seven measurements and entered them onto a prepared graph. The finished BGC was telephoned, faxed or e-mailed to our clinic or brought in at the time of the next re-evaluation.

2.8. Choice of food and insulin

Hills Kitten® or m/d® diet was used in the study. Compared with most other commercial cat foods, these diets are lower in carbohydrates (Kitten = 7.5%, m/d = 15.7%) and higher in protein (Kitten = 49.1%, m/d = 52.8%) and have a moderate amount of fat (Kitten = 34%, m/d = 19.4%) and a low to normal fiber content (Kitten = 0.6%, m/d = 6%) on a dry weight basis. If the cat would not eat either of these diets, another food was chosen and used consistently throughout the study. Caninsulin®[b] was used; it is an intermediate-acting insulin with 30 per cent crystalline and 70 per cent amorphous components of a porcine zinc insulin. The insulin was injected subcutaneously every 12 hours in all the cats.

2.9. Analysis of the data

The data were analyzed using nonparametric statistical methods.[f] The concentration of fructosamine, the mean values and fasting values of the BGCs done in the clinic (clinic curve) and at home (home curve), the insulin dose, the clinical signs and metabolic control before and after the reversal of the feeding time (relative to the insulin administration) were compared. The postprandial blood glucose concentration was calculated by determining the area under the glucose curve from the time of insulin injection until 6 hours later. The fasting blood glucose concentration was used as the basal value. Positive values indicated that the glucose concentrations increased during the 6-hour period, and negative values indicated a decrease in glucose concentrations. The faster the postprandial blood glucose values decreased, the more negative the value. The Wilcoxon signed ranks test was used to compare paired data and the Spearman rank-order correlation coefficient was used to measure the association between two variables. A P value ≤ 0.05 was considered significant. For graphical representation, scatter diagrams or scatter plots[g] were used; the latter consist of a vertical arrangement of measuring points and a horizontal line indicating the median.

3. Results

3.1. Means of the clinic curves

The mean values of the clinic curves ranged from 14.3 to 23.0 mmol/l (median 18.2 mmol/l) at time A, from 7.2 to 21.7 mmol/l (median 13.1 mmol/l) at time B, from 5.4 to 26.2 mmol/l (median 17.4 mmol/l) at time C and from 4.5 to 22.1 mmol/l (median 14.6 mmol/l) at time D. The mean values at times A were significantly greater than those at time B ($p = 0.05$). There were no significant differences among the means obtained at other times (A:C, A:D, B:C, B:D, C:D; Fig 1).

3.2. Means of the home curves

Of the eight owners of the cats that remained in the study until the end, six were able to carry out HM. In these six cats, the mean values of home curves ranged from 4.4 to 22.3 mmol/l (median 18.5 mmol/l) at time A, from 4.7 to 20.0 mmol/l (median 15.5 mmol/l) at time B, from 4.3 to 19.5 mmol/l (median 12.4 mmol/l) at time C and from 4.7 to 19.8 mmol/l (median 13.5 mmol/l) at time D. The mean values of the home curves for simultaneous insulin administration and feeding (A, B) did not differ significantly from those for times C and D, when an IMI of 45 minutes was used (Fig 3).

3.3. Fasting glucose concentration

The fasting glucose concentration (FGC) measured at home for the BGC, which was subsequently completed in the clinic, ranged from 7.8 to 33.3 mmol/l (median 23.6 mmol/l) at time A, from 15.2 to 31.8 mmol/l (median 22.8 mmol/l) at time B, from 5.8 to 32.2 mmol/l (median 18.6 mmol/l) at time C and from 7.4 to 27.0 mmol/l (median 22.4 mmol/l) at time D. There were no significant differences among values at the different time points. The FGC of the home curves ranged from 3.9 to 29.7 mmol/l (median 20.3 mmol/l) at time A, from 4.6 to 25.4 mmol/l (median 19.9 mmol/l) at time B, from 3.8 to 21.1 mmol/l (median 15.9 mmol/l) at time C and from 4.6 to 23.0 mmol/l (median 19.2 mmol/l) at time D. The FGCs at time A were significantly greater than those at time C ($p = 0.04$). The FGCs at times A and B (FGCs at time A and time B together) were significantly greater than those at times C and D ($p = 0.02$). There were no significant differences between the remaining time points before and after the change in feeding times (A:D, B:C, B:D; Fig 2 and 4).

3.4. Fructosamine concentration

The fructosamine concentration ranged from 434 to 826 µmol/l (median 601 µmol/l) at time A, from 347 to 791 µmol/l (median 576.5 µmol/l) at time B, from 354 to 788 µmol/l (median 544.5 µmol/l) at time C and from 376 to 722 µmol/l (median 589.5µmol/l) at time D. There were no significant differences among the values at times A, B, C and D (Fig 5).

3.5. Insulin dose

The insulin dose ranged from 0.11 to 0.82 U/kg (median 0.68 U/kg) at time A, from 0.11 to

1.23 U/kg (median 0.58 U/kg) at time B, from 0.22 to 1.22 U/kg (median 0.46 U/kg) at time C and from 0.11 to 0.82 U/kg (median 0.49 U/kg) at time D. There were no significant difference among the values at times A, B, C and D (Fig 6).

3.6. Body weight

The weights of the cats ranged from 4.3 to 7.5 kg (median 6.0 kg) at time A, from 4.4 to 7.6 kg (median 5.8 kg) at time B, from 4.2 to 7.4 kg (median 6.1 kg) at time C and from 4.2 to 7.3 kg (median 6.1 kg) at time D. There were no significant differences in weight among the different time points (Fig 7).

3.7. Metabolic control

The metabolic control score of the eight cats had a mean of 4.0 (moderate to poor) at time A, 4.5 at time B, 3.5 at time C and 4.5 at time D. The mean score of the metabolic control of all the cats was 4.0 during the entire study period. The scores for metabolic control before and after the feeding times were changed did not differ. The following table shows the scores for metabolic control in all the cats at the various time points.

Time	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5	Cat 6	Cat 7	Cat 8
A	4	4	2	4	5	5	6	3
B	3	5	1	4	6	4	5	5
C	2	3	1	6	6	5	4	3
D	4	4	1	5	5	6	5	3

3.8. Postprandial blood glucose concentration

The postprandial blood glucose concentration was determined in the six cats in which the blood glucose concentration could be monitored at home by the owners. Starting with the FGC, which was done at home and was followed by insulin administration and feeding, the area under the curve for 6 hours after insulin injection (3 blood glucose values) was determined. For the clinic curves, the postprandial blood glucose concentrations under the curve ranged from -63.2 to 51.2 h x mmol/l (median -48.7 h x mmol/l) at time A, from -93.2 to -33.8 h x mmol/l (median -58.0 h x mmol/l) at time B, from -110.8 to 54.0 h x mmol/l (median -28.4 h x mmol/l) at time C and from -100.6 to -23.2 h x mmol/l (median -58.2 h x mmol/l) at time D. There were no significant differences in the postprandial blood glucose concentrations of the clinic curves or the home curves before and after introduction of the IMI (Fig 8). There were no significant correlations between the fructosamine concentration and the postprandial blood glucose concentrations (expressed as area under the glucose curve), which were measured in the clinic or at home within a week prior to re-evaluation. There also was no significant correlation between the scores of metabolic control and the postprandial blood glucose concentrations.

3.9. Correlation between fasting glucose concentration and metabolic control

There was a significant correlation between the FGC of the clinic curves and the fructosamine concentration ($r=0.56$; $p=0.003$) as well as the metabolic control ($r=0.56$; $p=0.004$). The FGC

of the home curves was also correlated with the fructosamine concentration ($r=0.82$; $p<0.001$) and the metabolic control ($r=0.85$; $p<0.001$). Thus, the fructosamine concentration increased and the quality of metabolic control decreased with increases in the FGC (Fig 9).

4. Discussion

Based on our findings, the primary hypothesis that better metabolic control is achieved in diabetic cats by delaying the feeding time to 45 minutes after insulin administration was rejected. Compared with simultaneous insulin administration and feeding, an injection-meal interval of 45 minutes did not result in better metabolic control in the diabetic cats of our study. There were no significant differences in the mean and postprandial blood glucose concentrations of the BGCs, the fructosamine concentration, the insulin dose, the body weight and the clinical signs in the diabetic cats before and after changing the feeding time relative to the insulin administration. Although the FGC of the home curves one month after starting the 45-minute IMI were significantly lower than those when cats were given insulin and a meal simultaneously, there was no longer a significant difference two months later. Our results indicate that in diabetic cats, the feeding time does not have to be coordinated with the time of insulin administration. This had previously been postulated in a number of studies on feline diabetes mellitus on the basis that cats rarely have postprandial hyperglycemia that requires correction.[17,31,32] The last-mentioned statement is based on a recent study where the amount of food consumed and the postprandial blood glucose concentration were investigated in healthy and diabetic cats fed ad libitum. There was no increase in postprandial blood glucose concentration two hours after eating in any of the cats, and there was no correlation between the amount eaten and the blood glucose concentration. In that study, the cats were fed commercial dry and wet foods and raw meat.[18] However, other studies have shown that the blood glucose concentration after eating may indeed increase in healthy cats. A study on carbohydrate metabolism in cats found that starch in the diet did not significantly affect the postprandial blood glucose concentration compared with a control diet. In contrast, the blood glucose concentration increased markedly in cats that ate a high-glucose diet.[19] Although glucose is not a natural energy source for cats, it is often added to commercial feline diets in large amounts because of its high palatability.[33] Certain carbohydrates, such as rice, have a higher glycemic index than others, such as corn, and cause a greater increase in postprandial blood glucose concentration and insulin secretion in cats.[34] Other studies have shown that the composition of the diet has an important effect on postprandial blood glucose concentration. In healthy cats, a high-carbohydrate diet resulted in a 20 to 30 per cent higher peak in the postprandial blood glucose concentration compared with a diet high in protein and moderate in carbohydrate. In addition, the blood insulin concentration tended to be higher after a high-carbohydrate meal compared with a high-protein or high-fat meal.[35] Ad libitum feeding resulted in mean insulin concentrations that were at least 40% higher than in cats fed once daily, regardless of the type of diet.[20] These studies indicate that in cats, the concentrations of postprandial blood glucose and insulin are dependent on the amount of sugar and other carbohydrates in the diet. It is therefore very likely that there is also a relationship in diabetic cats between the composition of the diet, the feeding regime (ad libitum or once daily) and the blood glucose concentration. Based on the results of recent studies on diet composition for diabetic cats, we used low-carbohydrate, high-protein food (Hills Kitten and Hills m/d) in our study. The positive effect these diets have on metabolic control may favor a reduction in the dose of insulin or even discontinuation of insulin therapy.[14] It is quite plausible that a high-protein diet is also the best diet for diabetic cats because it results in a decrease in the concentrations of endogenous insulin and postprandial blood glucose in normal cats.[35]

Thus, the type of diet could have been the reason why metabolic control was not affected by the time of feeding in the cats of our study.
The feeding times that were defined at the start of the study did not satisfy the habits of all of the cats. Furthermore, some of the cats refused to eat the study diet at the beginning or even throughout the study period. Of the eight cats, two ate Hills Kitten® wet food, two others ate Hills m/d® dry food and one other Hills m/d® wet food. Of these five cats, three accepted one of the Hills diet only when a small amount of another commercial food was offered at the same time. The remaining three cats refused the two study diets and were fed another wet or dry food. Prior to the study, many of the cats were used to eating fresh food multiple times during the day and, during the study, did not eat the entire meal at once. Almost half of the cats had polyphagia and constantly begged for food during the study. Thus, maintaining the experimental feeding schedule was a challenge for the owners. Food was offered in the morning and evening at predetermined times and usually left out for the cat, which returned later to finish the meal. This feeding pattern is characteristic of cats. Studies on ad-libitum feeding showed that cats eat 12 to 20 small meals in a 24-hour period.[36] The feeding habits of diabetic cats do not differ from those of healthy cats[18] and we assume that this was the reason for the difficulties encountered with our feeding protocol.
There was no significant correlation between the postprandial blood glucose concentration and metabolic control in our study and consequently our secondary hypothesis was also rejected. Three of the eight cats received some of the insulin injections at the clinic where they often refused to eat. Obviously, this resulted in a distortion of the postprandial blood glucose concentrations in the clinic curves, but the curves generated at home, where the cats always ate their food, did not reveal a significant correlation between the two factors. In contrast, a close relationship between postprandial blood glucose concentration and metabolic control exists in human diabetics, in which maximal hyperglycemia occurs 60 to 120 minutes after eating.[27,37] Large meals result in higher postprandial glucose levels and require higher insulin doses for optimal control.[38] In cats that receive insulin every 12 hours, the highest blood glucose concentrations usually occur in the morning and evening before feeding. A previous study found that after eating and simultaneous administration of insulin, the blood glucose concentration of cats progressively decreased for 4 to 6 hours, regardless of the amount of food eaten, indicating that the shape of the BGC is affected primarily by the insulin and not by eating.[18] This is in agreement with the results of our study; the postprandial blood glucose concentration rarely increased above the fasting concentration but rather started to decrease immediately after administration of insulin.
The fasting blood glucose concentration was significantly correlated with both the fructosamine concentration and the quality of the metabolic control. The higher the fasting blood glucose concentration was in the morning, the poorer the cat's metabolic control. Similar findings have been reported in diabetic dogs. In a study of diabetic dogs, the concentrations of fasting blood glucose and fructosamine were significantly higher in 28 dogs with poor metabolic control than in 25 dogs with good metabolic control.[39] In human medicine, the fasting blood glucose concentration is one test used to differentiate healthy and diabetic patients or to determine whether a person is in a pre-diabetic phase.[3] However, using the fasting blood glucose concentration to assess metabolic control is not recommended in diabetic cats. Rather, metabolic control should be evaluated using BGCs, the fructosamine concentration and clinical signs.

In conclusion, the results of our study indicate that in diabetic cats, a 45-minute IMI does not improve metabolic control. There was no correlation between the quality of metabolic control and postprandial blood glucose concentration. However, there was a significant correlation between metabolic control and the fasting blood glucose concentration in the morning. In contrast to human diabetics, the course of the BGCs of diabetic cats was affected primarily by

the administration of insulin and not by eating. There is recent evidence suggesting that the composition of the diet plays a much more important role in the development of diabetes in cats than the feeding regime. Besides, considering the natural feeding habits of cats and the challenges faced by owners, a strict feeding regime for diabetic cats may not be realistic.

5. Footnotes

[a] Humalog®, Eli Lilly and Company, Indianapolis, USA
[b] Caninsulin®, Intervet, Boxmeer, the Netherlands
[c] Ascensia Elite®, Bayer Diagnostics, Zurich, Switzerland
[d] Microlet Vaculance®, Bayer Diagnostics, Zurich, Switzerland
[e] Cobas integra 700, Roche Diagnostics, Basel, Switzerland
[f] SPSS/PC V 11.0. base manual, SPSS Inc., Chicago, III
[g] GraphPad Prism 4, GraphPad Software Inc., San Diego CA, USA

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7. Graphs

Figure 1: Mean blood glucose concentrations of the clinic curves

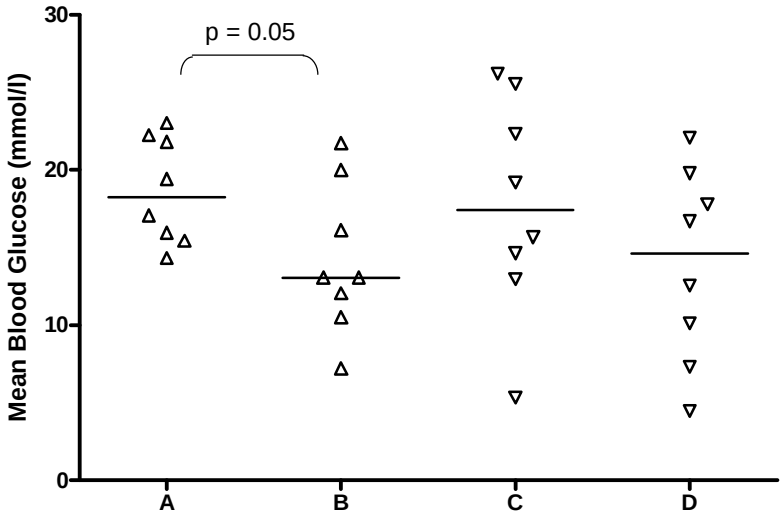
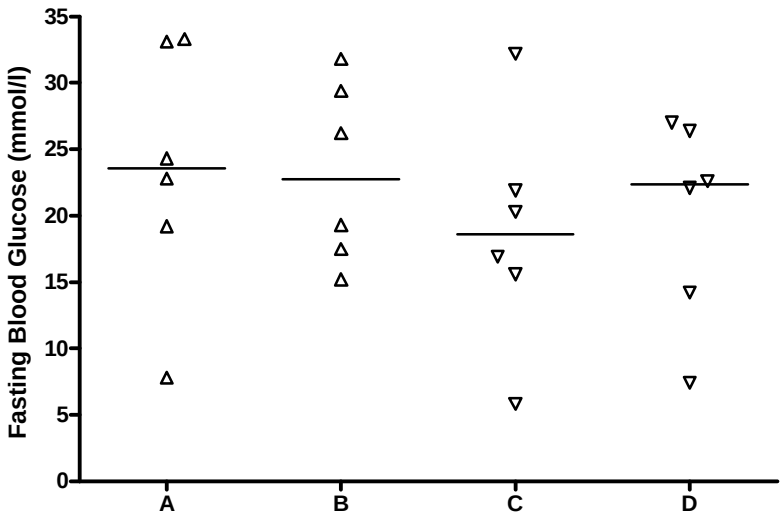


Figure 2: Fasting blood glucose concentrations of the clinic curves



Figures 1 and 2: Mean and fasting blood glucose concentrations of blood glucose curves generated in 8 diabetic cats in the clinic at the time of re-evaluation. Times A and B represent the period when simultaneous insulin administration and feeding were carried out, and times C and D represent the periods when a 45-minute insulin-meal interval was used. The fasting blood glucose concentrations were measured at home by the owner, and the remainder of the

measurements were carried out in the clinic. Re-evaluations were spaced 4 weeks apart. The means of the clinic curves at time B were significantly lower than those at time A (p=0.05).

Figure 3: Mean blood glucose concentrations of the home curves

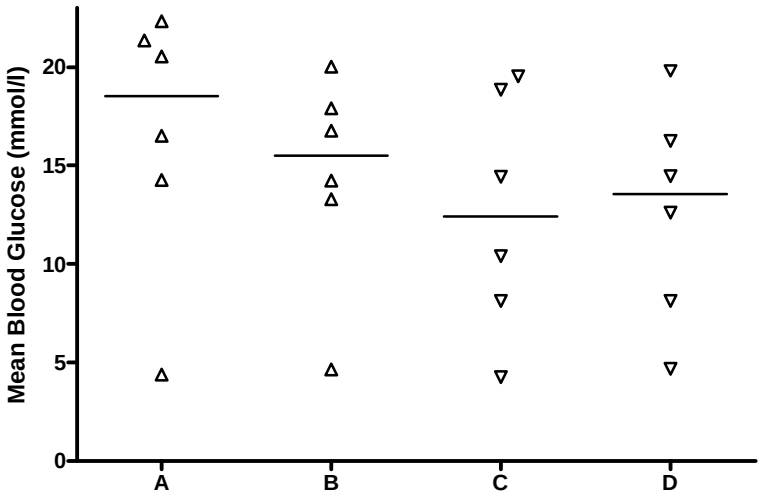
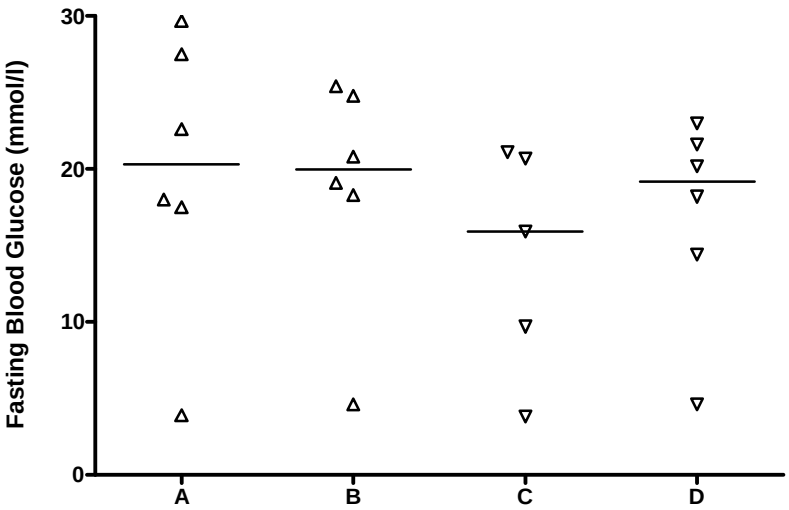


Figure 4: Fasting blood glucose concentrations of the home curves



Figures 3 and 4: Mean and fasting blood glucose concentrations in blood glucose curves generated at home in 6 diabetic cats. Times A and B represent the period when simultaneous insulin administration and feeding were carried out, and times C and D represent the periods when a 45-minute insulin-meal interval was used. Re-evaluations were spaced 4 weeks apart. The fasting blood glucose concentrations at time A were significantly higher than those at time C (p = 0.04).

Figure 5: Fructosamine concentration

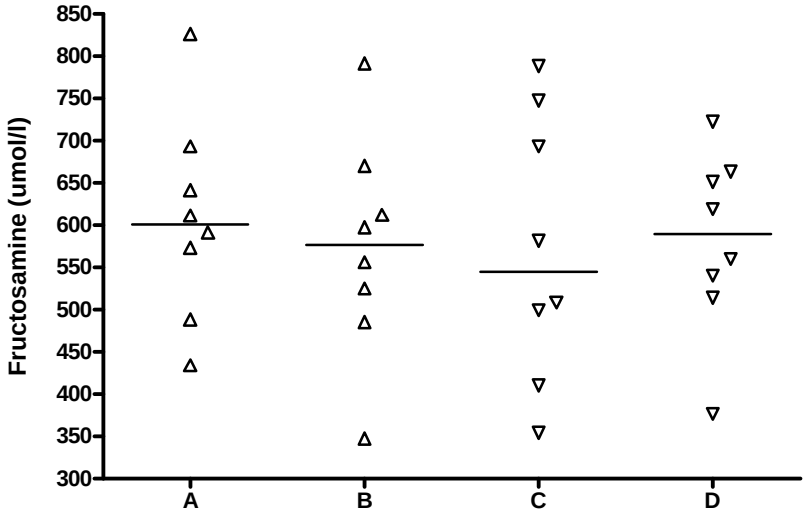


Figure 6: Insulin dose

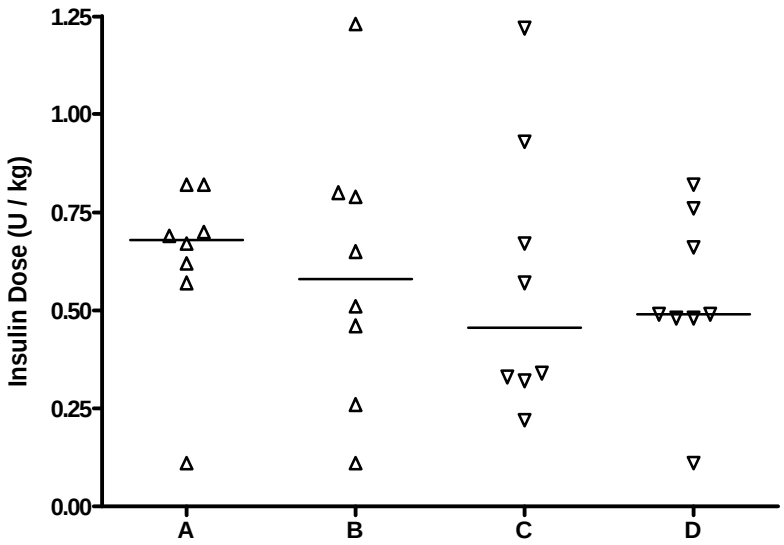
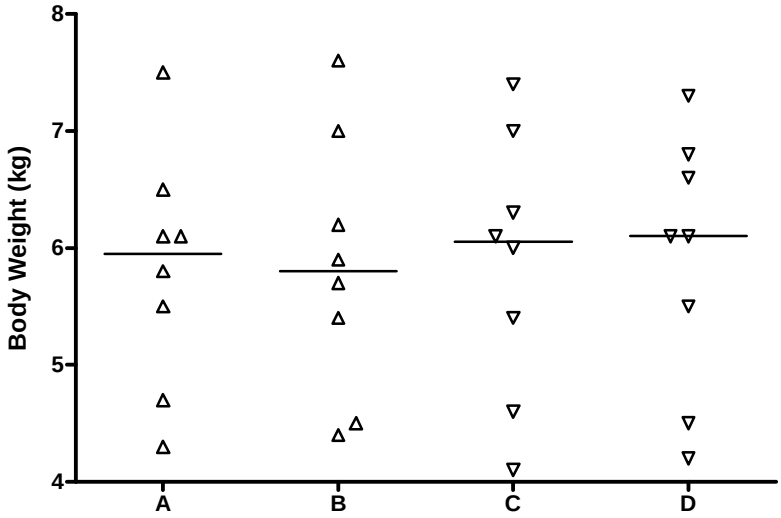
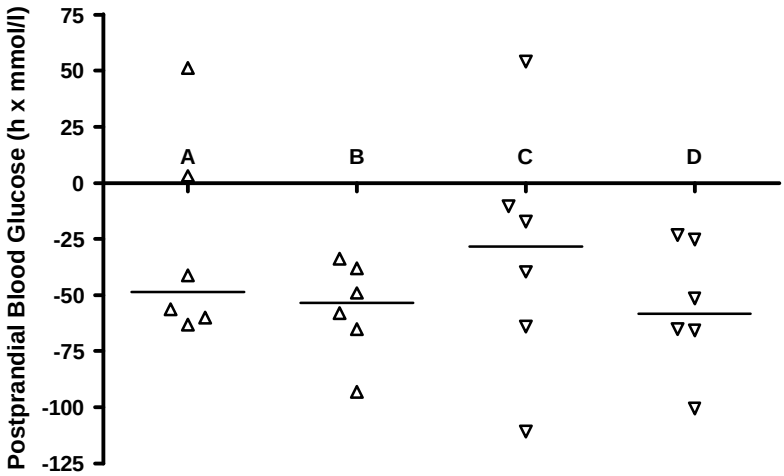


Figure 7: Body weight



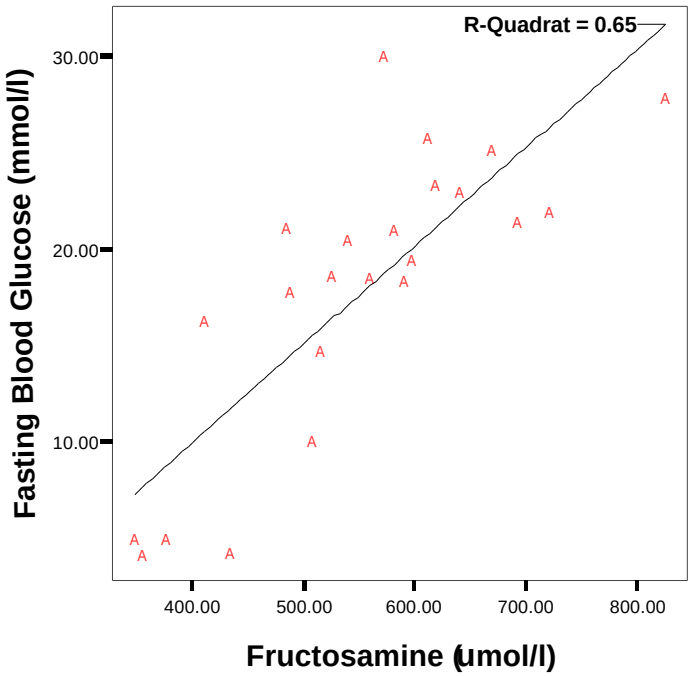
Figures 5 to 7: Concentration of fructosamine and insulin dose and body weight in 8 diabetic cats. Times A and B represent the period when simultaneous insulin administration and feeding were carried out, and times C and D represent the periods when a 45-minute insulin-meal interval was used. Individual re-evaluations were spaced 4 weeks apart. There were no significant differences among the means at the various time points.

Figure 8: Postprandial blood glucose concentration determined at the clinic



Postprandial blood glucose concentrations of blood glucose curves generated in 6 diabetic cats at the time of re-evaluation in the clinic. Times A and B represent the period when simultaneous insulin administration and feeding were carried out, and times C and D represent the period when a 45-minute insulin-meal interval was used. Re-evaluations were spaced 4 weeks apart. The postprandial blood glucose concentration was calculated as the area under the blood glucose curve; the fasting blood glucose concentration represented the basal value and the 6-hour value was the end-point. There were no significant differences among the values at times A, B, C and D.

Figure 9: Correlation between fasting blood glucose concentration and fructosamine concentration



The correlation between the concentrations of fructosamine and fasting blood glucose, determined in 6 diabetic cats during 23 re-evaluations 4 weeks apart. The fasting blood glucose concentration was measured at home and the fructosamine concentration was determined a few days later in the clinic. There was a significant correlation (p=0.001) and a correlation coefficient of 0.82.

Day-to-day variability of blood glucose concentration curves generated at home in cats with diabetes mellitus

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Abstract

Objective: To evaluate day-to-day variability of blood glucose curves (BGCs) generated at home and in the clinic.

Design: Prospective clinical study.

Animals: 7 cats with diabetes mellitus.

Procedure: BGCs generated at home (home curve) on two consecutive days and within one week in the clinic (clinic curve) were obtained on two separate occasions (parts 1 and 2). In each part insulin dose, amount and type of food were consistent for all 3 BGCs. Results of home curves were compared with each other and with the corresponding clinic curve.

Results: Differences between fasting blood glucose, nadir, time to nadir, maximum blood glucose and mean blood glucose during 12 hours, as well as difference between fasting blood glucose and nadir and AUC of home curves had high coefficients of variation. The differences between parameters of home curves were not smaller than those between home and clinic curves, indicating a large day-to-day variability in both home and clinic curves. Evaluation of the paired home curves led to the same theoretical recommendation for adjustment of insulin dose on 43%, and evaluation of home and clinic curves resulted in the same recommendation on 50% of occasions. 67% pairs of home curves in cats with good glycemic control and only 25% pairs of home curves in cats with poor glycemic control led to the same recommendation.

Conclusions and Clinical Relevance: There is considerable day-to-day variability of BGCs generated at home. Cats with good glycemic control may have more reproducible home curves than cats with poorer control.

Introduction

The measurement of blood glucose concentration and generation of blood glucose curves (BGCs) are commonly used during long-term management of cats with diabetes mellitus.[1] Until recently, BGCs were almost always carried out in a veterinary hospital because most cat owners are unable to collect blood samples by venipuncture. However, the procedure is time consuming and relatively expensive and therefore, not done as frequently as required. Additionally, stress or decreased food intake can markedly influence blood glucose concentrations. For these reasons, home monitoring of blood glucose concentrations was introduced a number of years ago.[2,3] Owners use an automatic-lancing device to collect a drop of capillary blood from the cat's ear, and the blood glucose concentration is determined using a portable blood glucose meter (PBGM). The values obtained for capillary blood correlate well with those of venous blood obtained from a peripheral vein.[4,5] Most owners of diabetic cats are willing and able to learn the technique of home monitoring, and long-term compliance is good.[6,7]

In human medicine, home monitoring has been used by diabetic patients since the late 1970s. Because the long-term complications of diabetes mellitus in humans can be greatly reduced by good glycemic control[8], home monitoring has become an integral part of effective treatment. However, several human studies have shown that the blood glucose concentration can vary markedly from day to day.[9-11] It is assumed that the fluctuations are due to variations in food intake, physical activity and emotional stress[10] and/or to variable absorption of injected insulin.[11] Day-to-day variations in blood glucose concentrations are also thought to occur in diabetic cats.[1] To date, there has been only one study on the reproducibility of blood glucose curves in cats with diabetes mellitus. In that study, blood glucose curves generated at home by the owner were compared with those done in the clinic. The differences between the home and clinic curves were substantial, and in almost 40% of the cats, the hypothetical treatment decisions derived from the 2 blood glucose curves did not agree.[7]

The goal of the present study was to investigate the day-to-day variability of home-generated BGCs in cats with diabetes mellitus. Our hypothesis was that there is better agreement between paired blood glucose curves generated at home than between curves generated at home and those generated in the clinic.

Materials and Methods

Selection of cats

Seven diabetic cats that ranged in age from 7 to 14 years (median age, 12 years) and weighed 4.3 to 7.5 kg (median weight, 6.1 kg) were included in this study. There were 5 castrated male and 2 spayed female cats, and breeds included domestic shorthair (n = 3), Persian (n = 1), Siamese (n = 1), Burmese (n = 1), and Carthusian (n = 1). The diagnosis of diabetes mellitus was based on characteristic clinical signs, fasting hyperglycemia, glucosuria and elevated serum fructosamine concentration (>340 µmol/l). Cats were included in the study provided that owners were willing to learn home monitoring, to return to our clinic for re-evaluations and to generate the required blood glucose curves at home. The cats had been diagnosed with diabetes mellitus 85 to 690 days (mean 252 days) prior to inclusion in the study. Treatment consisted of subcutaneous injection of a porcine intermediate-acting insulin[a] every 12 hours. The insulin dosage and quality of glycemic control varied among the cats during the study.

Home monitoring

The concept of home monitoring was introduced to owners at the time of diagnosis or at the

first evaluation at the clinic. One week after initial evaluation, cats were re-evaluated and home monitoring was again discussed. At a second re-evaluation 3 weeks later, owners were given the opportunity to learn the technique. This usually took a minimum of 30 minutes and consisted of repeated demonstrations of the use of the lancing device and PBGM. Owners then carried out the procedure once or twice on their cats. They were also taught how to calibrate the PBGM, check its accuracy by use of a control strip, and record blood glucose concentrations on prepared forms. In addition, owners received written and illustrated instructions for measuring blood glucose and generating BGCs. After instruction in the clinic, owners were asked to generate a BGC at least once every 3 to 4 weeks from that date. They were also asked to generate additional BGCs for the requirements of this study (see below). Collection of capillary blood from the ear was done as described[2,4,6,12] by use of a lancing device[b], and all blood glucose concentrations were measured with a commercially available PBGM[c]**.** At the time of this study all cat owners were able to perform home monitoring without difficulties.

Study design

The study consisted of two parts. In part 1, cat owners generated two BGCs on two consecutive days at home. Within 7 days of completion of the home BGCs, the cats were admitted to the clinic, where a third BGC was generated. For all BGCs, blood glucose was measured every 2 hours for 12 hours starting immediately before the morning insulin injection (total of 7 measurements per day). The insulin dose, time of insulin administration, and feeding regimen were constant for each cat in all three BGCs.

In part 2, this same protocol (two BGCs at home, one BGC in the clinic) was repeated a minimum of 4 weeks after completion of part 1. The insulin dose, time of insulin administration, and feeding regimen were constant for each cat in all three BGCs; however, they were not necessarily the same as in part 1. The collection of capillary blood and measurement of blood glucose concentration at home and in the clinic were done with the same lancing device and PBGM. The BGCs generated at home were referred to as home curves and BGCs generated in the clinic as clinic curves.

Besides generating BGCs, re-evaluations included a detailed updated history, physical examination, and measurement of hematocrit and concentrations of serum fructosamine, albumin and total protein. Serum fructosamine analyses were done by use of an automated analyzer[d] and commercial reagents supplied by the manufacturer. The cats were divided into 2 groups based on glycemic control. Group A consisted of cats that were considered to have good glycemic control; there was resolution or marked improvement in clinical signs and the serum fructosamine concentration was • 500 µmol/l. Group B consisted of cats that were considered to have moderate to poor glycemic control; there was persistence or little improvement of clinical signs and the serum fructosamine concentration was > 500 µmol/l.

Analysis of data

For each blood glucose curve the following seven parameters were determined: fasting blood glucose concentration, nadir, time to nadir, maximum blood glucose concentration, mean blood glucose concentration during 12 hours, difference between fasting blood glucose concentration and nadir (fasting-nadir), and area under the blood glucose curve (AUC). For parts 1 and 2, the differences between the 7 parameters were calculated for the following 3 pairs of curves: the 2 home curves, first home curve and clinic curve and second home curve and clinic curve. For each of the six pairs of curves, the mean, standard deviation (SD) and coefficient of variation (CV) were calculated for the differences between the corresponding parameters. A normality test carried out using StatView 5.1[e] revealed no significant

difference between the values and a normal distribution and therefore parametric tests were used. A paired *t*-test and one-way ANOVA were used to analyze differences between parts 1 and 2, between values of pairs of home curves and between values of pairs of a home curve and a clinic curve.[f] To identify significant sources of variation in the paired curves, a factorial ANOVA was carried out followed by a Bonferroni/Dunn post hoc test.[e] Differences were considered significant at $P \leq 0.05$. Scatterplots[g] were used for graphical representation of the data with a horizontal line showing the mean. To examine possible clinical implications of day-to-day variations, a theoretical recommendation was made for the adjustment of the insulin dose, which was based on the results of each blood glucose curve. The recommendation was to increase or decrease the insulin dosage or to leave it unchanged when the nadir was $\geq$ 9.0 mmol/l, < 5.0 mmol/l or 5.0 – 8.9 mmol/l, respectively.[7,13]
To determine possible causes of variability of the BGCs, the mean blood glucose concentration and nadir of blood glucose of each of the home curves were compared with the corresponding clinic curve in each cat. Thus, based on the mean and nadir, the clinic curve could be higher or lower than one or both of the home curves. The same criteria were used to compare the home curves.

Results

Comparison of differences between home and clinic curves

The differences among the 7 parameters from BGCs obtained on 3 days (2 home curves and 1 clinic curve) would be small if there was minimal day-to-day variability of the BGCs. However, the differences between the parameters were large for all pairs of BCGs. This was reflected by standard deviations of the differences that were almost as large as the means, and by large coefficients of variation, which ranged from 69% and 101% (Table 1). To test our hypothesis that there is better agreement between paired blood glucose curves generated at home than between curves generated at home and those generated in the clinic, the differences between the parameters of the home curves were compared with the differences between the parameters of the home and clinic curves. There were no significant differences, which meant that there was not greater agreement between the home curves than between the home and clinic curves, and that the hypothesis was to be rejected. The comparison of the differences that occurred between the paired home curves in parts 1 and 2 of the study showed that the difference between the maximum blood glucose concentration in part 2 was significantly higher than that in part 1($p=0.045$). There were no other significant differences (Fig. 1). There were also no significant differences between parts 1 and 2 with respect to differences that resulted from the comparison of the home and clinic curves and with respect to fructosamine concentrations.

Comparison of parameters of home and clinic curves

For these comparisons, absolute values of parameters of BGCs were used. The results of parts 1 and 2 were considered together, because the corresponding BGCs of the two parts (e.g. first home curve of part 1 and first home curve of part 2) did not differ with respect to any of the 7 parameters. The values of the fasting blood glucose concentration, maximum blood glucose concentration, mean blood glucose concentration and AUC were significantly lower in the first home curve than in the second home curve (Fig. 2). The fasting blood glucose and maximum blood glucose concentrations of the first home curve were significantly lower than those of the clinic curve. The nadir, fasting-nadir and time from insulin injection to nadir did not differ significantly between the first and the second home curve. The nadir, fasting-nadir, time from insulin injection to nadir, mean blood glucose concentration and AUC did not differ significantly between the first home curve and the clinic curve. There were no significant

differences between the second home curve and the clinic curve with respect to any of the 7 parameters.

Theoretical recommendations for adjustment of insulin dose

A total of 14 sets of paired home curves (7 from part 1 and 7 from part 2) were compared to determine a theoretical recommendation for adjustment of the insulin dose. For 46% (6 paired curves) of the paired curves, the same recommendations for adjustment of insulin dose resulted, and the theoretical recommendations varied for 57% (8 paired curves). In 6 of the latter 8 paired curves, there would have been no dose adjustment based on the results of one of the home curves and an increase or decrease in the insulin dose based on the results of the other home curve. In the remaining 2 paired curves, an opposite theoretical recommendation for insulin dose adjustment resulted; assessment of the results of one of the home curves led to a theoretical recommendation for a reduction in the insulin dose, whereas the results of the other home curve led to recommendation for an increase in the dose (Fig. 3a). There were 28 curve comparisons between home and clinic curves. Evaluation of the results of the home and clinic curves led to the same recommendation for adjustment of insulin dose in 14 cases (50%). In the other 14 cases, the recommendations differed: In 7 cases the results of the home curves indicated no adjustment, and an increase or decrease in the insulin dose would have been recommended based on the results of the clinic curves. In one case no adjustment of the insulin dose was made based on the clinic curve, whereas a reduction in the insulin dose was recommended based on the home curve. Evaluation of 6 paired curves led to opposite recommendations; in 3 cases, an increase in insulin dose was recommended based on the clinic curve and a decrease in insulin dose based on the home curve. In the other 3 cases, a decrease in the insulin dose was recommended based on the clinic curve and an increase based on the home curve (Fig. 3 b+c).

Quality of glycemic control

Two cats in parts 1 and 2, 1 cat in part 1 and another cat in part 2 were considered to have good glycemic control. Thus, when the home curves of parts 1 and 2 were considered together, there were 6 home curve pairs from cats with good glycemic control (group A) and 8 from cats with moderate to poor glycemic control (group B). In group A, 4 of the 6 home curve pairs (67%) led to the same theoretical recommendations for insulin dose adjustment and the remaining 2 home curve pairs (33%) led to different recommendations. In group B, the same recommendation for insulin dose adjustment was made in only 2 of 8 home curve pairs (25%); a different recommendation was made in the other 6 cases (75%). Of the 28 home curve and clinic curve pairs, 12 were from cats of group A and 16 from cats of group B. In group A, 8 (67%) of the 12 home and clinic curve pairs and in group B, 6 (38%) of the 16 home and clinic curve pairs led to identical recommendations for insulin dose adjustment. There were no significant differences between the 7 parameters obtained from the home curves of groups A and B, however, the differences between all of the parameters of group A tended to be smaller than those of group B (Fig 4).

Individual comparison of blood glucose curves

Comparison of home and clinic curves: In 2 cats, the clinic curves were higher than the home curves in both parts of the study. In 1 other cat, the clinic curve was considerably higher than the first home curve but corresponded to the second home curve. The mean and nadir of the clinic curves were considerably lower than those of the home curves in both parts of the study in 1 cat and in one part of the study in another cat. In the remaining 2 cats, the clinic curve corresponded to the home curve with the lower mean and nadir in both parts of the study. Comparison of the home curves: In 3 cats, the second home curve was much higher than the first; the difference was even more pronounced in part 2 of the study. In one cat the second

home curve was slightly higher than the first. In two cats the home curves were the same in both study parts. In the remainig cat the first home curve was slightly higher than the second curve in the first part and vice versa in the second part of the study.

Discussion

The results of the present study show that there is large day-to-day variability of blood glucose curves in diabetic cats even when factors such as insulin dose and meal size remain constant and the cat is at home in a stress-free environment. There was a large difference between the values of home curves obtained on two consecutive days. In particular, the maximum blood glucose concentration, the time from insulin injection to nadir and the fasting blood glucose concentration differed considerably between the two home curves. In contrast to our hypothesis, there was no greater agreement between the home curves than between the home and clinic curves. In insulin-dependent human diabetics, variations in blood glucose concentrations are known to occur within a 24-hour period as well as from day to day and are associated mainly with the activity level of the patient, meal composition and size, stress, and certain medications. [9-11,14] However, studies have shown that even when these factors remain constant, there is day-to-day variability of glucose concentrations. The causes include variable rate of absorption of insulin when different injection sites are used, variation in the length of insulin activity, variable insulin sensitivity among individuals, and variation in residual •-cell function.

In human diabetics, consistent SC insulin injection in the abdominal region results in faster absorption and smaller day-to-day fluctuations in blood glucose concentrations than rotating the injection site.[15] In the present study, owners varied the SC injection site regularly, which may have resulted in a variable rate of insulin absorption. This aspect has not been investigated in diabetic cats. The duration of action of the insulin[a] used in our study is about 12 hours, but may be shorter in some cats.[16] In the present study, the duration of insulin action was less than 12 hours in all three BGCs of part 1 in one cat. In fact the blood glucose concentration returned to baseline after 8 to 10 hours, which resulted in a marked difference in the morning fasting blood glucose concentration.

Because the majority of cats suffer from type 2 diabetes mellitus, the insulin dose depends on the severity of insulin resistance and the amount of residual •-cell function.[17,18] The degree of insulin sensitivity has a diurnal variation and also varies from day to day in human diabetics.[19,20] In healthy cats, there is also substantial day-to-day variability of insulin sensitivity, which may apply to diabetic cats as well, although this has not been investigated.[21]

In addition to internal factors, the reproducibility of the blood glucose concentration curves may have also been affected by external factors. The activity level of the cats was very difficult to control, and it possibly differed on the consecutive days of blood glucose measurement at home. Although the insulin dose remained constant, there may have been slight errors in the amount of insulin, drawn up by the owner.

In the individual comparison of home and clinic curves different patterns were observed: In the 3 cats in which the nadir and mean blood glucose of the clinic curves were higher than in one or both home curves we suspected stress-induced Hyperglycemia caused by hospitalization. In 4 cats the mean and nadir of the clinic curves were lower than both home curves respectively corresponded to the home curve with the lower mean and nadir. Three of these 4 cats did not eat or ate later in the day while hospitalized for the clinic curves. Possibly, these lower blood glucose values were attributable to reduced food intake in the clinic. Casella et al. (2005) reported that some of the variables of BGCs generated in the clinic in diabetic cats were significantly lower than those generated at home by the owner and were thought to be due to decreased food intake in the clinic.[7]

Two of the 3 cats in which the second home curve was much higher than the first in both study parts also had conspicuously high blood glucose concentrations in the clinic in one or both parts of the study, which was thought to be stress-induced. It is plausible that in these cats, frequent pricking of the ear was stressful, even at home. These cats had been subjected to home monitoring for several months but they had never undergone two consecutive days of blood collection prior to the study. Information on whether the cats were cooperative during blood collection was not available. However, fractiousness is not the only indicator of stress; some cats experience anxiety and stress while remaining cooperative and quiet.[22]
The fact that 3 of 7 cats had higher values in the second home curve than in the first led to significant differences in the statistical analysis: the fasting blood glucose concentration, maximum blood glucose concentration, mean blood glucose concentration, and AUC were significantly higher on the second day of home testing than on the first. This lends further support to the likelihood of stress-induced hyperglycemia on the second day of home testing. The nadir, time to nadir and difference between fasting blood glucose concentration and nadir are critical for evaluation of the insulin dose. The theoretical recommendation for insulin dose adjustment in the present study was similar to that of a recent study on cats with diabetes mellitus.[13] Comparison of the home curves led to the same recommendation on only 43% of occasions, and comparison of home and clinic curves resulted in the same recommendation on only 50% of occasions. Fleeman et al. (2003) investigated the reproducibility of BGCs in 10 diabetic dogs by comparing 3 pairs of BGCs generated on 2 consecutive days in the clinic. In that study, the theoretical recommendation for insulin dose adjustment was the same on 57% of occasions. In addition, 20 sets of paired curves from dogs with good glycemic control (nadir < 10 mmol/l) were compared and the theoretical recommendation was the same in only 35% of the curves.[23] This led to the conclusion that BGCs vary greatly from day to day particularly in dogs with good glycemic control. In contrast to the results of Fleeman et al. (2003)[23], there was better agreement between the BGCs of cats with moderate to good glycemic control in the present study; 6 of 14 pairs of home curves led to the same theoretical adjustment of insulin dose, and 4 (67%) of these were curves from cats with better glycemic control. In cats with moderate to poor glycemic control comparison of the home curves led to the same recommendation in only 2 of 8 (25%) paired curves. This suggests that there is less day-to-day variability of blood glucose concentrations in cats with good glycemic control. There was a non-significant trend for the differences between the parameters of the home curve pairs to be smaller in cats with good glycemic control. A larger number of diabetic cats or paired BGCs may have yielded more explicit results. Our results however, compare to those in humans. Molnar et al. (1972) described that the day-to-day variability of blood glucose concentration curves differed among healthy people and diabetics with good and poor glycemic control. Healthy humans had considerably less day-to-day variability of blood glucose concentrations than humans with diabetes mellitus, and human diabetics with poor glycemic control had significantly more variability than those with good glycemic control.[9]

Overall there was large day-to-day variability in blood glucose concentration curves in the diabetic cats of the present study. This is in agreement with dogs and humans with diabetes mellitus. Stress associated with hospitalization was thought to be a cause of elevated blood glucose concentrations in some of the cats although, blood glucose concentrations in the clinic may also be lower than those at home most likely due to lack of intake. The day-to-day variability between the home and clinic curves was not larger than that between paired home curves. The reproducibility of home curves in diabetic cats with good glycemic control tended to be better than that of cats with poorer control. We therefore assume that BGCs generated at home in cats with good glycemic control are more reliable than those in cats with poorer glycemic control. The results of this study also indicate that serial blood collection on two consecutive days may result in stress-related hyperglycemia in diabetic cats.

Footnotes

[a] Caninsulin®, Intervet, Boxmeer, the Netherlands
[b] Microlet Vaculance®, Bayer Diagnostics, Zurich, Switzerland
[c] Ascensia Elite®, Bayer Diagnostics, Zurich, Switzerland
The PBGM Ascensia Contour®, Bayer Diagnostics, Zurich, Switzerland was used in 1 of the 7 cats.
[d] Cobas integra 700, Roche Diagnostics, Basel, Switzerland
[e] StatView 5.1, SAS Inc., Wangen bei Dübendorf, Switzerland
[f] SPSS/PC V 11.0. base manual, SPSS Inc., Chicago, III
[g] GraphPad Prism 4, GraphPad Software Inc., San Diego CA, USA

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Tables

Table 1:

Comparison	Parameter	Min/max diff.	Mean diff.	SD of diff.	CV (%)
Home1 vs Home2	Fasting blood glucose (mmol/l)	0.2 - 23.1	7	6.9	98
	Nadir (mmol/l)	0.0 - 7.6	3	2.7	91
	Maximum blood glucose (mmol/l)	0.0 - 13.9	4.7	4.8	101
	Fasting-nadir (mmol/l)	0.1 - 16.9	5.4	5	92
	Mean blood glucose (mmol/l)	0.1 - 9.6	4.3	3.6	83
	Time to nadir (h)	0.0 - 4.0	1.6	1.6	99
	AUC (h x mmol/l)	1.0 - 115.2	50.7	43.4	86
Home1 vs Clinic	Fasting blood glucose (mmol/l)	0.8 - 14.6	5.6	4	72
	Nadir (mmol/l)	0.1 - 10.7	3.8	3.6	94
	Maximum blood glucose (mmol/l)	0.1 - 12.0	5.1	4.5	88
	Fasting-nadir (mmol/l)	0 0 - 15.2	4.4	4.3	96
	Mean blood glucose (mmol/l)	0.1 - 12.3	4.1	3.8	92
	Time to nadir (h)	0.0 - 4.0	1.9	1.4	76
	AUC (h x mmol/l)	4.3 - 141.9	50.8	46.5	92
Home2 vs Clinic	Fasting blood glucose (mmol/l)	0.5 - 10.5	5.1	3.5	69
	Nadir (mmol/l)	0.1 - 8.5	3.8	3.1	81
	Maximum blood glucose (mmol/l)	0.1 - 11.6	3.6	3.6	99
	Fasting-nadir (mmol/l)	0.6 - 12.3	4.9	4	81
	Mean blood glucose (mmol/l)	0.4 - 10.4	3.7	3.1	82
	Time to nadir (h)	0.0 - 4.0	1.9	1.7	89
	AUC (h x mmol/l)	3.9 - 126.8	44.3	38.2	86

Figures

Figure 1:

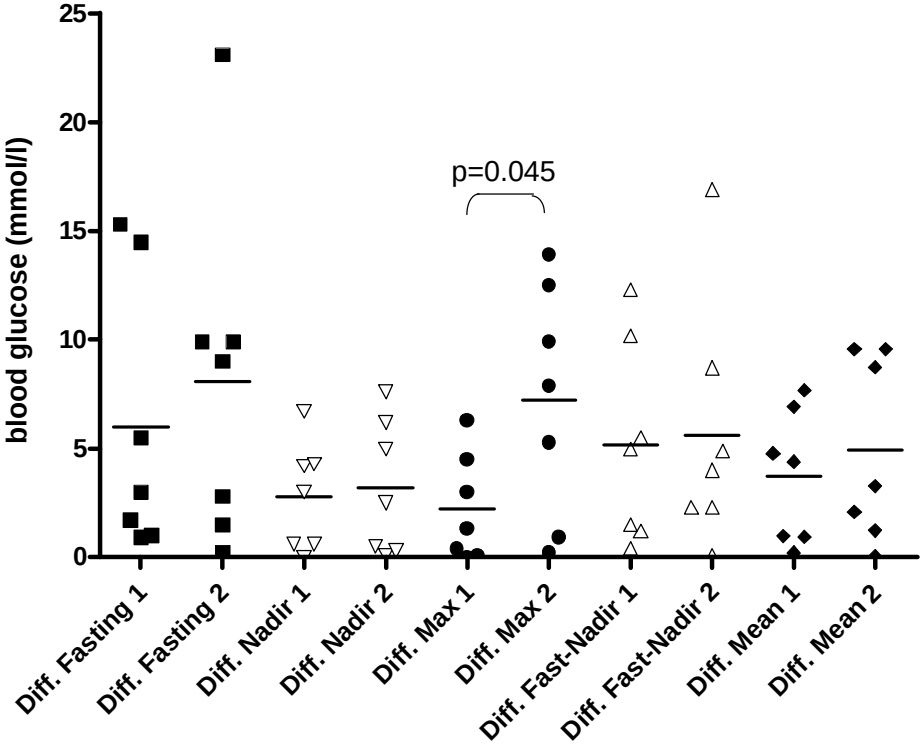


Figure 2:

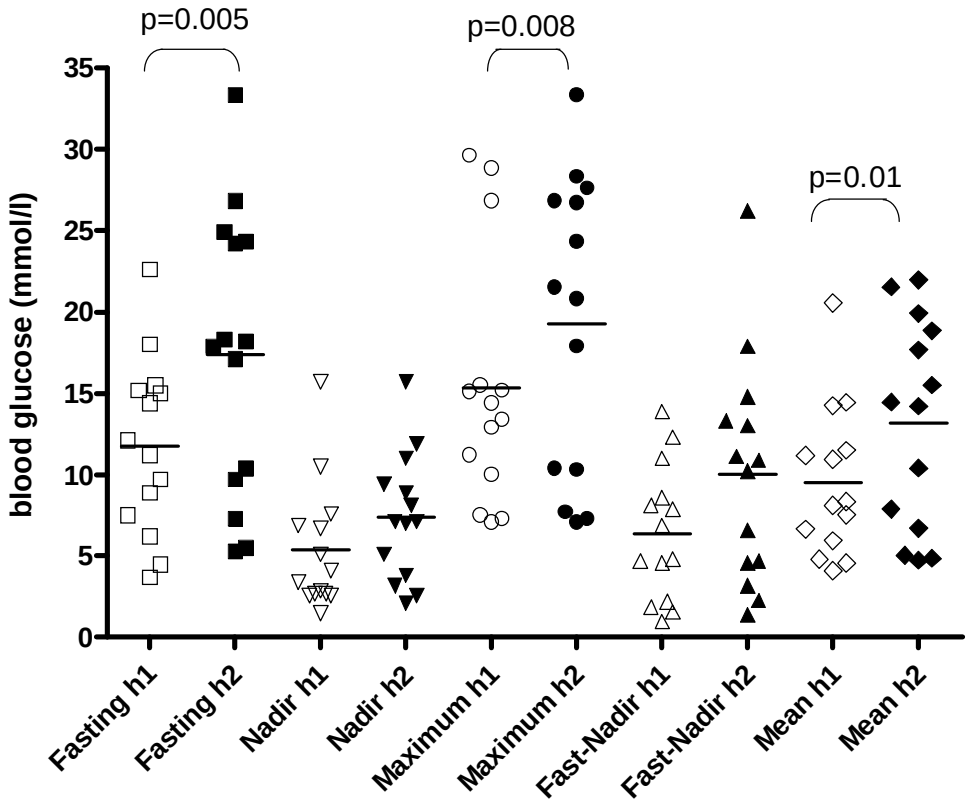


Figure 3a:

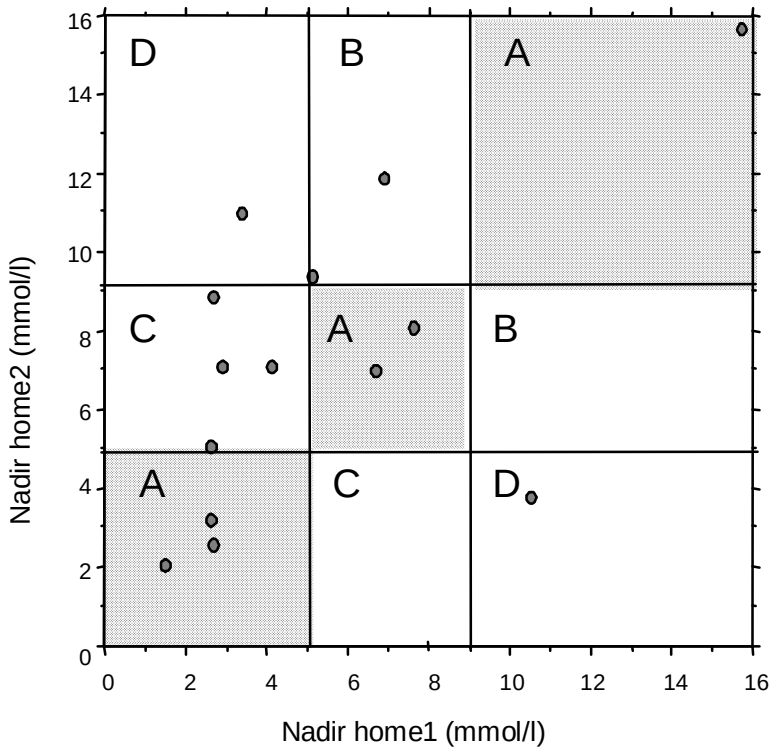


Figure 3b:

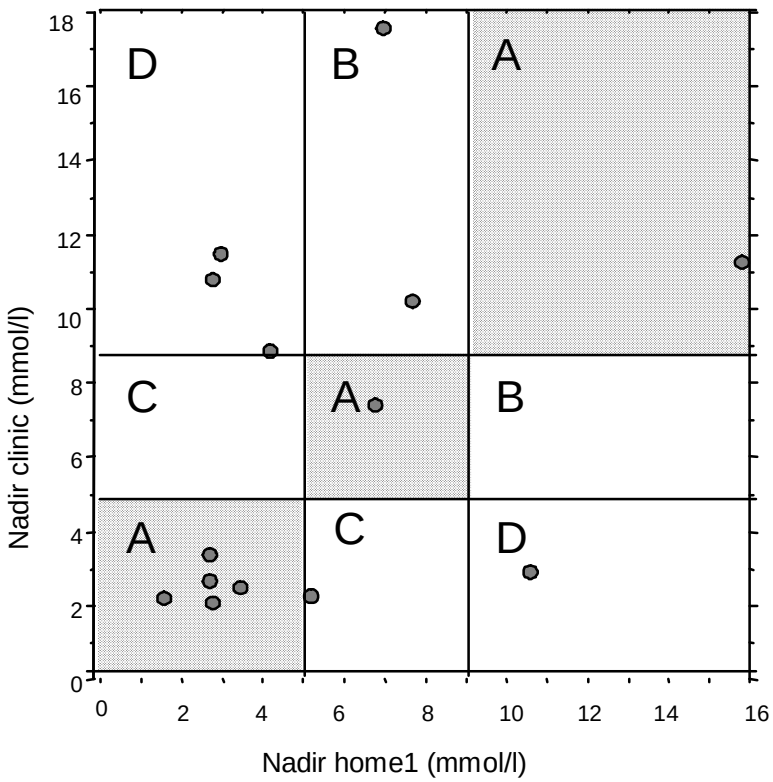


Figure 3c:

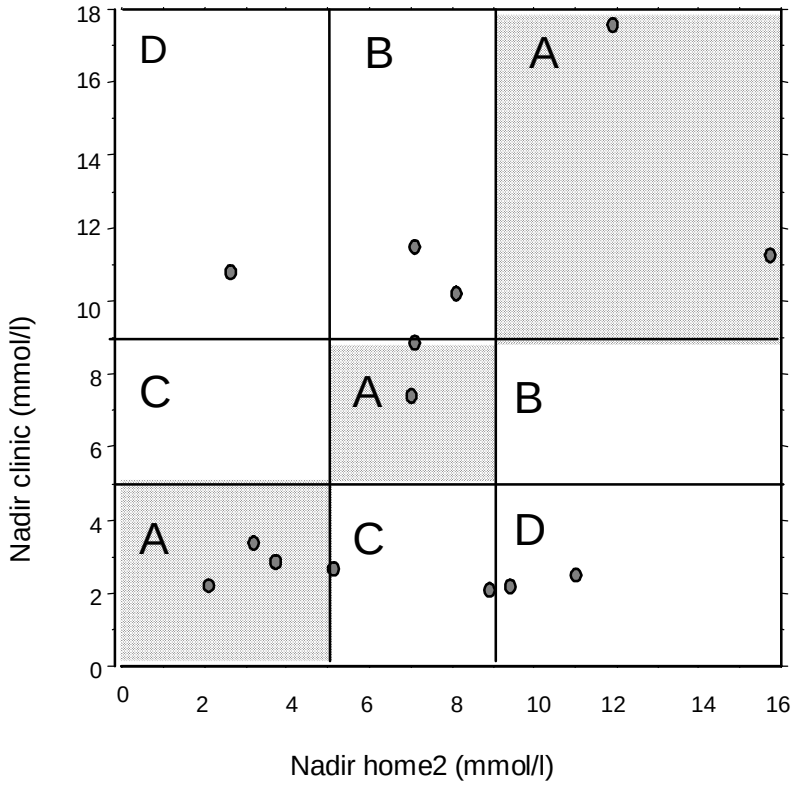
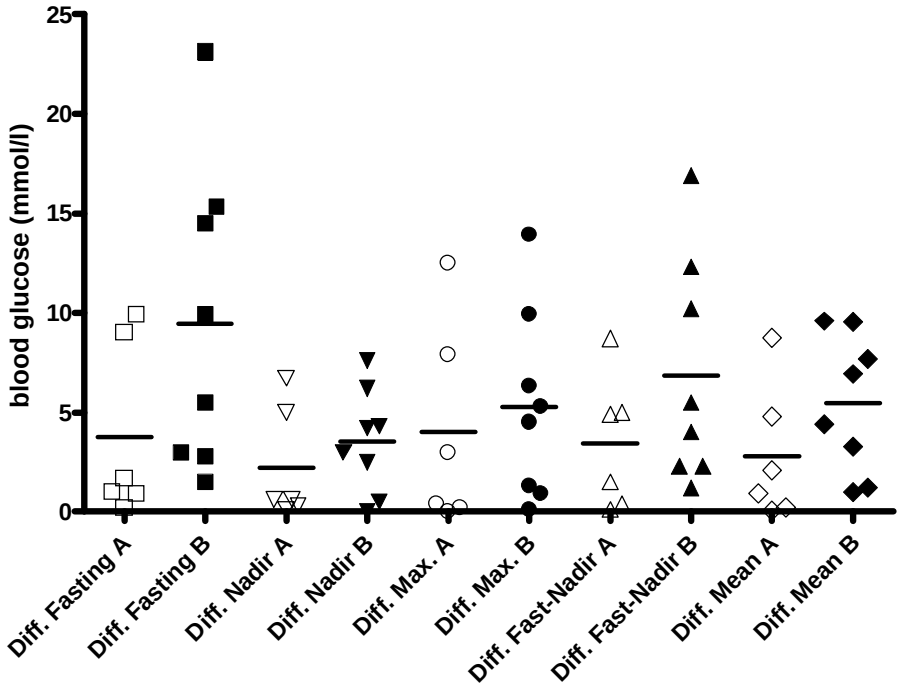


Figure 4:



Legends

Table 1: Parameters of blood glucose curves (BGCs) obtained from 7 diabetic cats in parts 1 and 2 of the study. In each part, three BGCs were generated for each cat: two at home (home 1 and home 2) and one at the clinic (clinic). The minimum and maximum difference, mean difference, standard deviation (SD), and coefficient of variance (CV) of difference between parameters were determined in each comparison between 14 paired curves.

Figure 1: Differences between fasting blood glucose concentrations (Diff. Fasting), nadir (Diff. Nadir), maximum blood glucose concentration (Diff. Max), fasting-nadir (Diff. Fast-Nadir), and mean blood glucose concentration (Diff. Mean) of paired home curves in 7 diabetic cats. In parts 1 and 2 of the study, 7 paired BGCs each were evaluated. Differences between maximum blood glucose of the two BGCs were significantly higher in part 2.

Figure 2: Fasting blood glucose concentration, nadir, maximum blood glucose concentration, fasting nadir and mean blood glucose concentration of home curves generated on two consecutive days (h1/h2) in 7 diabetic cats. Results of parts 1 and 2 of the study were combined resulting in a total of 14 values per parameter and day of measurement. Fasting blood glucose concentration, maximum blood glucose concentration and mean blood glucose concentration on the second day (h2) were significantly higher than on the first day (h1).

Figure 3a-c: Nadirs of two home curves (home 1, home 2) and one clinic curve (clinic) of 7 diabetic cats. Three BGCs were generated for each cat on two separate occasions (part 1 and part 2) resulting in a total of 14 measurements. The hatched panels A represent values from pairs of curves that both led to the same theoretical adjustment in insulin therapy. Panels B, C and D represent values from pairs of curves that led to different adjustments. In B and C one curve led to no change and the other to a decrease and an increase in insulin dose, respectively. In D an opposite adjustment was recommended; one curve led to an increase and the other a decrease in insulin dose. The drawn through lines in the square represent the nadir limits at 5.0 and at 9.0 mmol/l. The recommendation was to increase ore decrease the insulin dosage or to leave it unchanged when the nadir was • 9.0 mmol/l, < 5.0 mmol/l or 5.0-8.9 mmol/l, respectively.

Figure 4: Differences between fasting blood glucose concentration, nadir, maximum blood glucose concentration, fasting nadir and mean blood glucose concentration of two BGCs generated at home on two consecutive days in 7 diabetic cats. The consecutive BGCs were repeated on two separate occasions (part 1 and part 2), and a total of 14 pairs of curves were evaluated. Six pairs of curves were obtained from cats with good glycemic control (group A) and 8 pairs of curves from cats with moderate to poor control (group B). For all parameters, differences tended to be lower in cats with moderate to good glycemic control, but the differences were not significant.

Evaluation of IGF-1 levels in cats with transient and permanent diabetes mellitus

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Abstract

It was investigated if IGF-1 levels in cats which experience diabetic remission (i.e. transient diabetes mellitus) differ from those in cats with permanent disease. Thirteen of 32 diabetic cats showed remission within 16 weeks after initiating insulin therapy, nineteen cats continued to need insulin therapy. IGF-1 concentrations were measured before (t_0), 1 – 3 (t_1) and 4 – 8 (t_2) weeks after initiating insulin therapy.

No difference in IGF-1 levels was found between cats with transient and permanent diabetes at any point in time.

In both groups of cats IGF-1 concentrations were significantly lower compared to those of controls before insulin administration. After starting insulin therapy IGF-1 increased significantly in both groups. In cats with transient diabetes IGF-1 levels were not different from controls already at t_1, whereas in cats with permanent diabetes it took until t_2.

Although IGF-1 levels seem to normalize faster in cats with transient diabetes mellitus measurement is not helpful to predict the course of the disease.

Keywords

cat – diabetes mellitus – IGF-1 – diabetic remission

Introduction

The growth hormone (GH)-insulin-like growth factor (IGF-1) axis is an integral part of the endocrine system responsible for promoting linear growth. Insulin is a major anabolic effector in the body, and is also an important regulator of the GH-IGF axis (Bereket *et al* 1999). It is well known that in humans with poorly controlled or untreated type 1 diabetes mellitus IGF-1 concentrations are frequently low which is assumed to be due to low portal insulin levels. Decreased IGF-1 levels in turn cause GH hypersecretion via reduced negative feedback at the hypothalamus and pituitary level, exacerbating insulin resistance and thus establishing a vicious circle of raised GH and poor glycemic control (Donckier 2003). Less is known about the GH-IGF-1 axis in humans with type 2 diabetes mellitus. IGF-1 levels have been reported to be decreased or normal (Tan and Baxter 1986, Frystyk *et al* 1999). It has been assumed, that the degree to which IGF-1 is decreased in human type 2 diabetes reflects the degree of beta-cell impairment, e.g. insulin secretory capacity (Kratzsch *et al* 1996).

Very little is known about the GH-IGF-1 axis in cats with diabetes mellitus. In one study it was shown that untreated diabetic cats had significantly lower IGF-1 levels compared to control cats. IGF-1 levels increased after insulin therapy was initiated and were not different from those of controls after 4 to 8 weeks. GH concentrations did not change during insulin therapy (Reusch *et al* 2001, Reusch *et al* 2006). Another study showed that mean IGF-1 levels in short-term diabetic cats were significantly lower than those in normal cats (Starkey *et al* 2004).

In 20 – 40% of diabetic cats remission occurs after about 1 to 3 months of insulin therapy. This phenomenon, called transient diabetes is thought to be due to the recovery of beta cells from glucose toxicity (Rand 1999). The exact mechanisms are unknown so far, it may be assumed however, that for remission to occur a sufficient number of beta-cells has to be present. Up till now there are no clinical parameters to predict if the course of the disease will be transient or permanent. It is also not known, if portal insulin concentrations differ between cats with a transient or permanent course of the disease.

The goal of the present study was to investigate if IFG-1 levels in cats with transient diabetes mellitus differ from those in cats with permanent disease.

Materials and Methods

Cats with diabetes mellitus

Thirty two cats with diabetes mellitus were enrolled in the study. They ranged in age from 4 to 16 years (median 10) and weighed 2.5 to 9.8 kg (median 5.5). There were 17 castrated male and 15 spayed female cats. Breeds included 29 domestic shorthair cats, 2 persian cats and one siamese.

A diagnosis of diabetes mellitus was based on clinical signs (e.g. polyuria, polydipsia, weight loss), hyperglycemia (fasting blood glucose > 9mmol/l) and elevated fructosamine concentrations (> 340 µmol/l). Cats with diabetic ketoacidosis and those with acromegaly were excluded from the study. All cats were followed for a minimum of 16 weeks after diagnosis. For further analyses the cats were grouped as follows:

Group 1 Cats with permanent diabetes mellitus

Nineteen cats which continued to be diabetic and received insulin therapy during the duration of the study.

Group 2 Cats with transient diabetes mellitus

Thirteen cats with transient diabetes mellitus. In those cats clinical signs of diabetes mellitus disappeared, blood glucose and fructosamine normalized and insulin therapy was

discontinued within 16 weeks after starting therapy.

Control cats
IGF- 1 levels of the diabetic cats were compared to those of previously examined control cats. In eighteen healthy cats IGF-1 levels ranged between 196.0 and 791.0 ng/ml (median 452.0) (Reusch *et al* 2006).

Study design
Blood specimens for IGF-1 and routine laboratory testing were obtained from all animals by venipuncture. All diabetic cats received intermediate-acting insulin (Caninsulin^R, Intervet NL; Lente MC^R, Novo Nordisk DK) twice daily from the time of presentation at our clinic.
In diabetic cats IGF-1 measurements were performed prior to insulin therapy at initial presentation (t_0), 1 – 3 weeks (t_1) and 4 – 8 weeks (t_2) after starting insulin therapy. In healthy cats IGF-1 levels were determined once.

Analytical procedures
IGF-1 concentrations were determined by radioimmunoassay after chromatography of the serum samples on silica cartridges (SepPak C18, Waters Corp. Milford, MA) as described previously (Reusch *et al* 2006). Briefly, 0.15 ml PBS/0.2% human serum albumin (pH 7.4) was added to 0.1 ml cat serum, and the mixture was acid treated and run on Sep Pak cartridges according to the protocol supplied by Immunonuclear (Stillwater, MN). After reconstitution with 1 ml PBS/0.2% serum albumin samples were assayed at 3 different dilutions (undiluted, 1:2, 1:4), rhIGF-1 was used as standard. The radioimmunoassay was performed as described (Zapf *et al* 1981).
Fructosamine analyses were performed on a Cobas Integra 700 (Roche, Basel, Switzerland) analyzer using commercial reagents (Fructosamine, Roche, Basel, Switzerland). Complete blood counts, serum biochemical analyses and urinalyses were performed using standard laboratory methods.

Statistical analysis
Results were analyzed by means of nonparametric statistical methods (SPSS/PC V 10.0. base manual, SPSS Inc. Chicago, Ill). Ranges and median values are reported. Differences within groups were tested using Friedman ANOVA and the Wilcoxon signed rank test for matched pairs, to test differences between groups the Mann-Whitney-U-test was used. Correlations were tested using the Spearman rank-order correlation coefficient. For all analyses, a value of $P \leq 0.05$ was accepted as significant.
Box-and-whisker plots were used to show the distribution of the data. The whiskers represent the range and the box represents the 25th and 75th percentiles of the data. The horizontal line in the box marks the median.

Results

IGF-1 concentrations
In cats with permanent diabetes mellitus (group 1) IGF-1 ranged between 45-543 ng/ml (median: 220) at t_0, 175-616 ng/ml (median: 303) at t_1 and 151-748 ng/ml (median: 337) at t_2.
In cats with transient diabetes mellitus (group 2) IGF-1 concentrations were 54-593 ng/ml (median: 262) at t_0, 204-750 ng/ml (median: 457) at t_1 and 152-973 ng/ml (median: 437) at t_2 respectively.
In both groups IGF-1 concentrations were significantly lower at t_0 compared to t_1 and t_2. No difference was found between concentrations at t_1 and t_2.

IGF-1 levels did not differ between the two groups at t_0, t_1 and t_2.
Both groups had significantly lower IGF-1 levels compared to controls at t_0. At t_1 IGF-1 in group 1 was still significantly lower, whereas levels in group 2 did not differ from those in controls. At t_2 IGF-1 levels in both groups did not differ from those in controls.
Fig. 1 shows IGF-1 levels in controls and group 1 and group 2 at t_0, Fig. 2 shows IGF-1 levels in groups 1 and 2 at t_0, t_1 and t_2.

Blood glucose and fructosamine concentrations and insulin dosages
In group 1 fasting blood glucose and fructosamine concentrations did not differ between t_0 and t_1 and were significantly lower at t_2 compared to t_0. In group 2 both parameters were significantly lower at t_1 and t_2 compared to t_0.
At t_0 fasting blood glucose and fructosamine concentrations did not differ between the two groups, whereas at t_1 and t_2 both parameters were significantly lower in group 2 compared to group 1.
In group 1 insulin dosages significantly increased and were significantly higher at t_1 and t_2 compared to t_0, and significantly higher at t_2 compared to t_1. In group 2 insulin dosages significantly decreased and were significantly lower at t_1 and t_2 compared to t_0, and significantly lower at t_2 compared to t_1. Insulin dosages (in U/kg body weight) at t_0 did not differ between the two groups, whereas at t_1 and t_2 they were significantly lower in group 2.
Ranges and median values of fasting blood glucose, fructosamine and insulin dosages at the different points in time are given in table 1.

Discussion

The finding of this study that cats with diabetes mellitus have significantly lower IGF-1 levels prior to insulin therapy compared to healthy cats confirms the results of a previous study (Reusch *et al* 2006). So far, only those two studies investigated IGF-1 levels in untreated diabetic cats. The results however, were not unexpected since in humans and other species as rat and dog low IGF-1 levels have been demonstrated in case of diabetes mellitus (Eigenmann *et al* 1977, Scheiwiller *et al* 1986, Bereket *et al* 1999).
Another research group recently studied IGF-1 levels in cats which had been treated with insulin for various time periods (Starkey *et al* 2004). Cats with short term treatment (31 days and less) had significantly lower IGF-1 levels than controls, whereas no difference to controls was revealed in cats with a treatment duration of 32 days to 14 months. Although our re-evaluations were done at shorter intervals those results roughly compare with ours. After 4 to 8 weeks of insulin administration IGF-1 levels had normalized in cats with permanent as well as with transient diabetes mellitus.
In humans, IGF-1 concentrations are known to be closely related to insulin levels in the portal circulation. Low portal insulin levels lead to GH resistance through down regulation of hepatic GH receptors (Donckier 2003). There are conflicting data on IGF-1 levels during insulin therapy. Some authors state, that with the peripheral mode of insulin administration portal insulin levels remain inadequate and IGF-1 levels therefore low (Hanaire-Broutin *et al* 1996, Dunger and Acerini 1998). Others describe a normalisation of IGF-1 in a time-dependent manner after starting insulin therapy (Bereket *et al* 1995).
In cats the exact mechanism for the low IGF-1 levels prior to insulin treatment is unknown so far. Due to the results of the previous (Reusch *et al* 2006) and the present study, in which a close association between treatment with insulin and IGF-1 increase was demonstrated, we hypothesize that the mechanisms are similar.
In cats with transient diabetes mellitus IGF-1 levels were normal already after 1 to 3 weeks of insulin treatment, whereas in cats with permanent diabetes mellitus IGF-1 levels were still

significantly lower than those of control cats. This finding can not be explained by differences in the amount of exogenous insulin (e.g. higher insulin dosages in cats with transient diabetes). At this point in time cats with transient diabetes received significantly less insulin than cats with permanent diabetes. We assume that in the cats with transient diabetes beta-cells regained some function soon after initiating exogenous insulin therapy, which allowed portal insulin levels to increase. Currently it is thought that recovery from the effects of glucose toxicity is the underlying mechanism for a transient course of diabetes mellitus in cats. Glucose toxicity is defined as impaired insulin secretion from beta-cells as a result of prolonged hyperglycemia. Initially, the glucose-mediated insulin suppression is functional and reversible. Later, structural changes occur, which, with time become irreversible, lead to beta-cell loss (Yki-Jarvinen 1992, Rand 1999). Glucose toxicity is the reason why measuring insulin concentrations prior to therapy is neither helpful to define if the disease will have a transient course, nor to predict which cat will respond to oral hypoglycaemic drugs (Nelson *et al* 1999, Rand 1999, Tschuor *et al* 2006). According to one study the same seems to hold true for measuring insulin during glucagons tolerance testing (Nelson *et al* 1999). Prior to insulin therapy we did not find a difference in IGF-1 levels between cats with transient and with permanent diabetes mellitus. On the background of the above mentioned facts one might argue that this had to be expected because glucose toxicity leads to suppression of insulin secretion, which leads to low portal insulin concentration resulting in low IGF-1 levels.
It is of interest however, that insulin concentrations in the portal vein are higher than in the peripheral circulation, in humans and dogs a difference of factor 5 has been demonstrated (Song *et al* 2000). Therefore we initially had speculated, that cats with transient diabetes would eventually have sufficient residual beta-cell function and a sufficient portal insulin level to guarantee normal IGF-1 production.
In summary IGF-1 levels are not different in cats with a transient or a permanent course of diabetes mellitus prior to insulin administration. After starting therapy IGF-1 levels may normalize faster in cats with transient than in cats with permanent diabetes. Measurement of IGF-1 at the time of diagnosis or during re-evaluations does not appear to be helpful to predict the course of the disease.

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Tables

Table 1: Laboratory paramters and insulin dosage

	Fasting blood glucose (mmol/l)			Fructosamine (µmol/l)			Insulin dosage (U/kg BID)		
	t0	t1	t2	t0	t1	t2	t0	t1	t2
Group 1	14.6-34.7 (24.5)	10.7-39.5 (21.6)	2.8-34.9 (19.8)	415-785 (637)	401-805 (563)	321-782 (532)	0.07-0.6 (0.34)	0.07-0.71 (0.48)	0.13-0.89 (0.53)
Group 2	9.8-41.0 (25.9)	3.6-38.0 (9.1)	3.8-18.4 (6.1)	371-893 (706)	292-626 (503)	277-564 (359)	0.1-0.35 (0.2)	0.05-0.44 (0.13)	0-0.24 (0.09)

Figures

Fig. 1: IGF-1 measurement in cats with diabetes mellitus and healthy controls

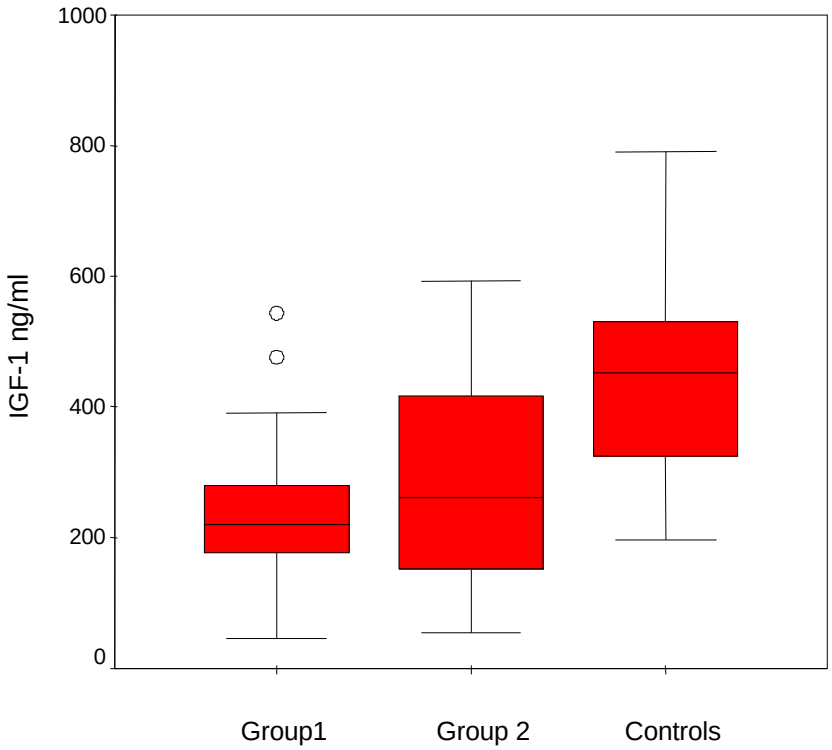
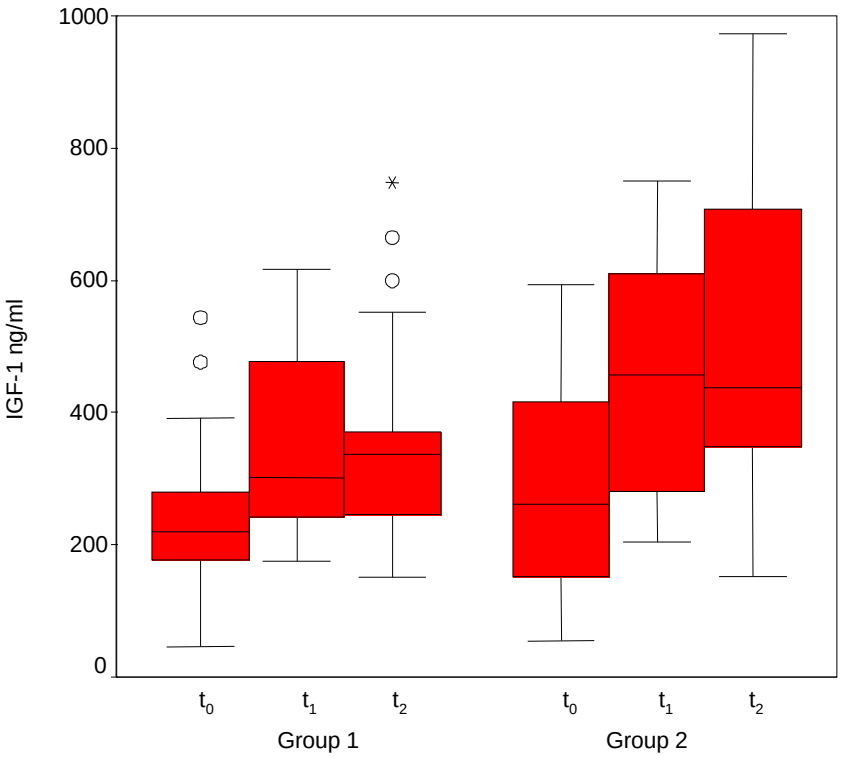


Fig. 2: IGF-1 measurement at different times



Legends

Table 1:
Ranges and median values of fasting blood glucose concentrations, fructosamine concentrations and insulin dosage at t_0, t_1 and t_3 in cats with permanent (group 1) and transient diabetes (group 2).

Fig. 1:
IGF-1 concentrations in cats with permanent (group 1) and transient (group 2) diabetes mellitus at the time of diagnosis. IGF-1 concentrations of control cats from a previous study are given for comparison (Reusch *et al* 2006). IGF-1 in both groups with diabetes were significantly lower compared to controls.

Boxes represent the 25^{th} and 75^{th} percentiles, the horizontal line represents the median and the whiskers represent the range. O = outlyer

Fig. 2:
IGF-1 concentrations in cats with permanent (group 1) and transient (group 2) diabetes mellitus before and after start of insulin therapy.
t_0 = prior to insulin therapy
t_1 = 1 – 3 weeks after start of insulin therapy
t_2 = 4 – 8 weeks after start of insulin therapy
In both groups IGF-1 concentrations were significantly higher at t_1 and t_2 compared to t_0.

Box plot see Fig. 1. O = outlyer, $*$ = extreme values

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